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REGULAR ARTICLE

HPTLC QUANTIFICATION OF FLAVONOIDS, LARVICIDAL AND SMOKE REPELLENT ACTIVITIES OF *CASSIA OCCIDENTALIS* L. (CAESALPINIACEAE) AGAINST MALARIAL VECTORE *ANOPHELES STEPHENSI* LIS (DIPTERA: CULICIDAE)

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SUMMARY

A simple High Performance Thin Layer Chromatographic (HPTLC) method has been developed for the analysis of flavonoid in ethanol extracts of *Cassia Occidentalis*. The amount of flavonoid in the extracts has been estimated by comparing the peak area using the standard. The proposed HPTLC method was found to be simple, faster and reliable for analysis of flavonoid. *Cassia Occidentalis* were the dominant invasive weed in the campus of Bharathiar University India. Their allelopathic activity has greatly affected the phytodiversity in the campus. With the view of their huge biomass prospecting, the larvicidal potential of ethanol extract of *Cassia Occidentalis* was tested against the larvae of *Anopheles Stephensi*. The ethanol extract of *Cassia Occidentalis* were found most effective with LC50 value of 60.69%, 64.76%, 67.78%, 70.56%, 92.21% of I, II, III, IV and pupa respectively. The smoke toxicity was more effective against the *Anopheles stephensi*. Smoke exposed gravid females oviposited fewer eggs when compared to those that were not exposed.

Key words: HPTLC, Larvicid, Smoke Repellent, *Cassia Occidentalis*, *Anopheles Stephensi* Lis

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1. Introduction

The mosquito is the principal vector of many of the vector-borne diseases affecting human beings and other animals. Mosquitoes constitute a major public health problem as vectors of serious human diseases (El Hag *et al*., 1999). In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia (Kumar et al., 2007). The control of mosquito larvae is dependent on regular applications of organophosphates and different insecticides (Yang *et al*., 2002). The major drawback with the use of chemical insecticides is that they are non-selective and could be more harmful to other non-target organisms. Moreover, after few years the mosquitoes develop resistance against the insecticides due to frequent use of them (Severini *et al*., 1993; WHO, 1970). Plants, being a natural source of various compounds, are known to contain larvicidal agents, which may act in combination or independently, hence necessiting to carry out the studies on the interactions of compounds (Gershenzon and Dudareva 2007; Wink 1993).

Many studies on plant extract against mosquito larvae have been conducted around the world. The leaf extract of *Datura metal* was reported to be toxic to *Spotoptera litura* (Murugan *et al*., 1999); The interactive

effect of botanicals (Neem, Pongamia) and Leucas aspers, *Bacillus sphericus* against the larvae of *Culex quinquefasiatus* (Murugan *et al*., 2003) .The methanol extract of *Sphaeranthus indicus* showed macrofilaricidal activity by worm motility and subsequent mortality was observed (Nisha *et al*., 2007). *Abutilon indicum* (Rahuman *et al*., 2008) were tested against the larvae of *A. aegypti, A. stephensi*, and *C. quinquefasciatus*. Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols and aldehydes act on mosquitoes in the vapor phase (Brown, 1977). These volatile compounds were effective against mosquitoes for a relatively short period, typically 15 min to 10 h (Barnard, 2000). The most promising botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Labiate, Meliaceae, Oocystaceae and Rutaceae (Sukumar *et al*., 1991). The repellent constituents are mainly monoterpenoids such as geraniol, citronellol, linalool, terpineol and carvone (Vartak and Sharma, 1993).

In the past few decades compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses (Dalziel, 1948; Abo et al., 1999; Hennebelle et al., 2009). Besides, they have been found to exhibit anti-inflammatory (Chidume et al., 2001), antioxidant (Yen et al., 1998; Yen and Chuang, 2000), hypoglycaemic (Bhakta et al., 1997; Jalalpure et al., 2004), antiplasmodial (Iwalewa et al., 1990, 1997), larvicidal (Yang et al., 2003), antimutagenic (Silva et al., 2008; Yadav et al., 2010) and anticancer activities (Prasanna et al., 2009; Yadav et al., 2010). They are also widely used for the treatment of wounds (Bhakta et al., 1998), skin diseases such as ringworm, scabies and eczema, gastro intestinal disorders like ulcers (Dalziel, 1948; Benjamin, 1980; Abo et al., 1999; Elujoba et al., 1999; Jacob et al., 2002), and jaundice (Pieme et al., 2006).The seeds are brewed into a coffee-like beverage for asthma, malaria, fevers and stomach complaints. In Africa the plant is used for abscesses, bile complaints, birth control, bronchitis, bruises, cataracts, constipation, edema, eye infections, fever, headache, increasing perspiration, inflammation, jaundice, kidney infections, leprosy, malaria, pain (kidney), menstrual disorders, rheumatism, ringworms, scabies, skin diseases, sore throat, stomach ulcers, stomachache, swelling, syphilis, tetanus, worms (Dalziel 1937; Keay 1958; Kerharo and Adam, 1974; Adjanohoun *et al*., 1985; Burkill 1997).The present paper is to investigate the effect of methanolic extract of *C.occidentalis* on the larvicidal , smoke repellent against malarial vectore *Anopheles stephensi*

2. Materials and Methods

Plant Material

Cassia occidentalis L.(Caesalpiniaceae) was collected from our Department garden, Bharathiar University, Coimbatore, India.

Preparation of plant extracts

Cassia occidentalis leaves were washed with tap water and shade dried at room temperature. The dried plant materials were powdered by an electrical blender. From the powder 200g of the plant material were extracted with 2.5 liters of organic solvents (ethanol) for 8 hrs in a soxhlet apparatus (Vogel, 1978). The crude plant extracts were evaporated to dryness in rotary vacuum evaporator.

HPTLC Quantification in Test Samples

Samples

Sample A – Ethanol extract

RUT – Rutin standard as a reference compound

Test solution preparation

The ethanol extract of *Cassia occidentalis* 100mg was weighed in an electronic balance (Afcoset) and dissolved in 1ml of Ethanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

Sample application

0.5µl of test solution and 3µl of standard solution (3mg in 10ml methanol) was loaded

as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Flavonoids) and the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.

Derivatization

The developed plate was sprayed with respective spray reagent (Flavonoids) and dried at 100°C in Hot air oven. The plate was photo-documented in Day light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning

After derivatization, the plate was fixed in scanner stage and scanning was done at UV 366nm. The Peak table, Peak display and Peak densitogram were noted.

Mobile phase

Ethyl acetate-butanone-formic acid-water (5:3:1:1)

Spray reagent

1% ethanolic aluminum chloride reagent and dried at 100°C for 2 minutes.

Detection

Yellow coloured fluorescent zones at UV 366nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the Presence of Flavonoids in the given sample.

Colonization of *Anopheles stephensi*
Collection of eggs

The eggs of *A.stephensi* were collected from National Institute for Communicable Diseases (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without expose to any insecticide and in and around Coimbatore, India at different breeding habitats with the help of a 'O' type brush. The eggs were then brought to the laboratory and transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml water and kept for larval hatching. They were hatched and reared have been still maintained from many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae

The freshly hatched larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae transformed into the pupae stage. The larvae reared in plastic cups. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing 500 ml of water with help of a sucker. The pupae containing glass beaker were kept in 90 x 90 x 90 cm size mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained 27.2°C, 75 - 85% RH, under 14L: 10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

Blood feeding of adult *Anopheles stephensi* and egg laying

The females were fed by hand every alternate day at 6.00 p.m. feeding mosquitoes on human arm for experimental purposes

was suggested by Judson (1967) and Briegel (1985). Both females and males were provided with 10% glucose solution as described by Villani *et al*. (1983) on cotton wicks. The cotton was always kept moist with the solution and changed every day. Theoder and Parsons (1945) noticed that glucose as well as ordinary sugar appeared equally attractive to the mosquitoes. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arranged made collection of eggs easier.

Preparation of required plant extracts concentration

One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations were prepared ranging from 20% to 100%.

Determination of median lethal concentration

LC50 (lethal concentration 50%) is the concentration of any toxic substance reducing by mortality the number of tested individuals to 50% in a prefixed time (Ravera, 1986). According to Rand and petrocelli (1985) the LC50 (median lethal concentration) is estimated to produce mortality in 50% of a test solution over a specific period of time. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of *A. stephensi* larvae to *Cassia occidentalis* for 24h. Determining LC50 concentration separate glass beakers of 500 ml of water capacity were taken. Then, different concentrations of *Cassia occidentalis* were added to different glass beakers. Then, 25 *A. stephensi* larvae were introduced into each glass beaker. A control beaker with 500 ml of water and 25 larvae were also maintained. The mortality/survival of larvae in the treatment beakers was recorded after every 24 h. the concentration at which 50% mortality of larvae occurred after 24h was taken as the medium lethal concentration (LC50) for 24 h. The LC50 concentration for 24 h. was calculated by the probit analysis method of Finney (1971).

Test for larvicidal and pupicidal activity

A laboratory colony of *A.stephensi* larvae were used for the larvicidal activity. Hundred numbers of first, second, third and fourth instars larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts were added. Larval food was given for the test larvae. In each concentration five replicates were tested. The control was set up by mixing 1ml of acetone with 249 ml of dechlorinated water. The control mortalities were corrected by using Abbott's formula (Abbott's, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larve introduced}} \times 100$$

The values of LC50, LC90 and their 95% confidence limit of upper confidence limit(UCL) and lower confidence limit(LCL) , regression and chi-square values were calculated by using probit analysis (finney,1971).

Pupal toxicity test

A laboratory colony of *A.stephensi* pupae were used for pupicidal activity. Twenty five numbers of freshly emerged pupae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1 ml of desired concentrations of plant extract was added. Five replicates were set up for each concentration and control was setup by mixing 1ml of acetone with 249 ml of dechlorinated water. The control mortality was corrected by Abbott's formula (Abbott's, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of Pupae introduced}} \times 100$$

The values of LC50, LC90 and their 95% confidence limit of upper confidence

limit(UCL) and lower confidence limit(LCL), regression and chi-square values were calculated by using probit analysis (finney,1971).

Smoke toxicity test

Cassia occidentalis leaves were used for smoke toxicity assay. The mosquito coils were prepared by following the method of Saini *et al.* (1986) with minor modifications by using 2.5 gram of plant ingredients, 4 grams of coconut shell and charcoal powder as burning material. These ingredients were thoroughly mixed with distilled water to form a semisolid paste. A Mosquito coil (0.6 cm thickness) was prepared manually and shade dried. The control coils will be prepared by without the plant ingredient.

The experiments were conducted in glass chamber measuring 140 X 120 X 60 cm. A window measuring 60 X 30 cm was situated at mid bottom of one side of the chamber. One hundred three or four day's old blood starved adult female mosquitoes were released into the chamber and were provided with 10% sucrose solution. A belly shaven pigeon was kept tied inside the cage in immobilized condition. The experimental chamber was tightly closed. The experiment was repeated five times on separate days including control groups using mosquitoes of same age. The data were pooled and average values were subsequently used for calculations. Control was maintained in two sets. One set was run with coil lacking the active ingredient of plant powder (control I) another one was a commercial coil (control 2), which was used for positive control to compare the effectiveness of plant coils. After the experiment was over, the fed, unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *A. stephensi* was calculated in terms of percentage of unfed mosquitoes due to treatment.

$$= \frac{\text{Number of unfed mosquitoes in treatment} - \text{Number of unfed mosquito in control 1}}{\text{Number of mosquito treated}} \times 100$$

The live blood fed mosquitoes were reared in a mosquito cage, measuring 30 x 30 x 15 cm. The top and bottom of the cage were fit with glass and all other sides were covered with muslin cloth. Water soaked raisins and a 5% sucrose solution soaked in cotton balls were provided as a food source. Water containing powdered yeast and dog biscuits were also kept inside the cage in a glass bowl for oviposition. The eggs from the cage were collected daily till all the mosquitoes died. A total 50- 100 eggs were allowed to hatch in plastic trays measuring 30 x 25 x 6 cm, containing about 2.5 liters of unchlorinated tap water. Hatched larvae's were fed with a mixture of dog biscuits and yeast powder in the ratio of 2:1 and water in the tray was changed daily. Survival and dead instars were counted and reduction in the population from the smoke treated mosquitoes was calculated using the formula.

$$\text{Population reduction} = \frac{\text{No. of larvae hatched in control 1} - \text{No. of larvae hatched in treated}}{\text{No. of larvae hatched in control 1}} \times 100$$

Statistical analysis

Larvicidal and pupicidal bioassay , percent control mortalities were corrected by using Abbots formula (Abbott's, 1925). Lc_{50}, Lc_{90} were calculated from toxicity data by using profit analysis (Finney, 1971).Data from larval and pupal mortality, ovicidal activity and repellent activity were subjected to analysis of variance (ANOVA). Stastical software SPSS were used for data analysis.

3. Results

Larvicidal and pupicidal activity of ethanol extract of *S.campanulata* leaf extract at various concentrations against malarial vector, *A.stephensi* is given in the Table 1. The plant extract exhibited larvicidal activity to different instars (I, II, III, and IV) of *A.stephensi.* The LC_{50} and LC_{90} values of *C.occidentalis* for I instar larvae of *A.stephensi* were 60.69 %, 119.74%, II instar 64.76 %, 121.60%, III instar 67.78 %, 123.35%, IV instar 70.56 %, 122.81% and pupa 92.21%,162.52%, respectively. The chi-square values were significant at 5 % level. Among the different larval stages, the I instar larvae was more susceptible than the other instar larvae.

Table1: Larvicidal activity of *Cassia Occidentalis* extract against different instars and pupae of malarial vector *Anopheles stephensi*

Larval Stages	% of larval mortality (%)								95% Confidence limit				Chi-Square value
	Concentration (%)					LC_{50}	LC_{90}	Regression Equation	LCL		UCL		
	20	40	60	80	100				LC_{50}	LC_{90}	LC_{50}	LC_{90}(%)	
I	20	34	48	60	85	60.69	(119.74)	Y=-1.31732 +0.02170X	55.20	108.54	66.25	136.03	3.371
II	15	30	43	58	85	64.76	(121.60)	Y=-1.55247 +0.02397X	59.78	108.09	70.00	132.53	3.797
III	15	28	40	54	83	67.78	(123.35)	Y=-1.56324 +0.02306X	62.62	112.29	73.40	139.21	4.787
IV	12	26	34	54	82	70.56	(122.81)	Y=-1.73098 +0.02453X	65.62	112.36	76.05	137.57	4.979
Pupa	10	18	26	39	58	92.21	(162.52)	Y=-1.68074 +0.01823X	84.15	142.42	103.72	195.25	0.698

Chi square value Significant at P <0.05 level

Table 2: Smoke toxicity effect of leaves of *C. Occidentalis* against biting activity of *Anopheles stephensi*

C.occidentalis parts used in grams	No. of mosquito tested	Fed mosquito	Unfed mosquitoes		Total	% unfed over control I
			Alive	Dead		
Leaf 2 G	100	20	32	48	80	63
Pods 2G	100	23	42	35	77	60
Control I	100	83	17	0	17	0
Control II	100	10	41	49	90	73

Control I: Negative control – blank without plant material
Control II: Positive control – mortein coil

Table 2 provides the results of smoke toxicity effect of *C.occidentalis* leaf on *A. stephensi*. Two gram of plant ingredients from the plant leaf and pods was used for the smoke toxicity. The control was maintained without plant ingredients. It acts as negative control. The commercially available (Mortein) mosquito coil used as positive control. One hundred 3-4 days starved *A. stephensi* larvae were used. After the individual treatment of each plant, the fed and unfed mosquitoes were counted. There were 20 fed and 80 unfed mosquitoes counted after the treatment of *C.occidentalis* leaf was counted. The comparisons of positive control with the plant product showed very high efficacy, but the plant products alone showed good smoke toxicity effect on *A. stephensi.*

Table 3: Smoke toxicity effect of leaves of *C.Occidentalis* on reproduction and survival of *Anopheles stephensi*

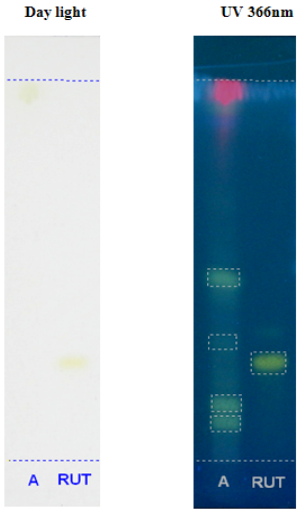
C.occidentalis parts used	No. of mosquito tested	Total No. of eggs	Total No of larva hatched from the eggs	% of reduction in population over control I
Leaf	25	793	342	74.40
Pods	25	747	320	76.04
Control I	25	1430	1336	-
Control II	25	820	267	80.01

Control I : Negative control – blank without plant material
Control II : Positive control – mortein coil

Table 3 shows the result of smoke toxicity effect of different parts of (leaves and pods) *C.occidentalis* ensured population of *A.stephensi*. The numbers of eggs laid by the alive, fed females were shown. Number of eggs laid and the hatchability were greatly reduced or affected by the exposure of smoke from *C.occidentalis*. The percentage reduction of hatchability by the smoke from leaves showed 74.40 % and from the pods showed 76.04%. The leaves showed a significant effect on the fecundity and hatchability.

The lethal effects observed on mosquito larvae could be attributed to the allelochemicals present in *C.occidentalis*, which were analyzed by HPTLC method. About eight different types of allelochemicals were detected in *C.occidentalis* (Table 4, Figure 1 and 2). The range of Rf values of these compounds was between 0.11 to 0.97. The compound detected in peak no. 8 (Figure 2) with height 83.9, area 1608.4% and Rf 0.97 (Table 4), was the major group of allelochemicals in *C.occidentalis*. This was followed by the flavonoids denoted by peak nos.1, 2, 4 and 5, while peak no.6, 7 denoted the least amount of compounds.

Fig. 1: HPTLC plates of *C. occidentalis* derivatised in different reagents



A-Ethanol extract of *Cassia Occidentalis*
Rut- Rutin standard

Fig. 2: Ethanol extract of *C.Occidentalis* Peak densitogram display (Scanned at 366nm)

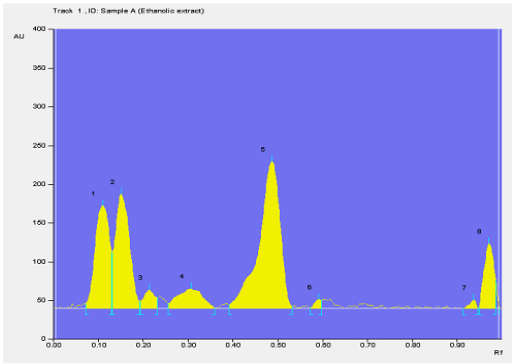


Table 4: R_f Values and Area of flavonoids in *C.occidentalis*

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.11	132.9	3857.0	Flavonoid 1
Sample A	2	0.15	147.8	4257.8	Flavonoid 2
Sample A	3	0.21	23.8	541.0	Unknown
Sample A	4	0.31	25.3	1290.1	Flavonoid 3
Sample A	5	0.49	189.8	8044.5	Flavonoid 4
Sample A	6	0.59	11.7	158.2	Unknown
Sample A	7	0.94	11.0	134.2	Unknown
Sample A	8	0.97	83.9	1608.4	Unknown
RUT	1	0.27	541.7	17490.8	Rutin standard

4. Discussion

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by preventing mosquito bite using repellent, causing larval mortality and killing mosquito's. At present, the main threat to effective mosquito control is resistance to insecticide in the mosquito (Chandra et al. 1998). Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources (Prabhakar and Jabanesan 2004). In addition, the use of medicinal plants for mosquito control is likely to generate local employment, reduce dependence on expensive imported products, and stimulate efforts to enhance public health (Bowers et al. 1995).

The effect of ethanol extract of *C.occidentalis* was studied in a dose dependent manner. The ethanol of *C.occidentalis* was found to have higher rate of larvicidal rate against *A. stephensi*, the concentration of extract have to be increased for better larvicidal effect. Senthilnathan (2007) observed that higher larvicidal effect of *Eucalyptus tereticornis* oil (leaf extract) with increased doses on *Anopheles stephensi*. He also observed that first and second instar larvae were most susceptible to all treatments. The larvicidal activity of four flavonoid compounds exhibits remarkable effect against larvae of *A. aegypti*. Cheng et al. (2004) reported larvicidal effect against *A. aegypti* larvae in four compounds derived from the essential oils of cinnamon leaf, including cinnamaldehyde, cinnamyl acetate, eugenol and anethole, with LC50 values of 29, 33, 33 and 42 mg/l, respectively. Our earlier report of mosquitocidal compound octacosane, which isolated from leaves of *Moschosma polystachyum* exhibited larvicidal activity with LC50 value of 7.2 mg/l against *Culex quinquefasciatus* (Rajkumar and Jebanesan 2004).

In the present study also the *C.occidentalis* ethanol extract showed larvicidal activity against the malarial vector *A. stephensi* at a dose equivalent to LC50 ranging between 60.69 %, 64.76 %, 67.78 %, 70.56 %, and 92.21%, for I, II, III, IV instar larvae and pupa respectively and previous literature of smoke toxicity Smoke emerged from *Albizzia amara* and *Ocimum basilicum* considerably affect the mosquito survival and pronounced high repellent potential (Murugan et al. 2007).

Anophalas karwari was repelled by coconut husks, ginger and betel nut leaves (Vernede and Marnix, 1994). In Soloman Islands, a recent survey revealed that fire with coconut husks and papaya leaves was the most prevalent form of personal protection from mosquitoes, being used by 52% of residents (Dulhunty *et al*., 2000). Number of eggs laid and hatchability of *Anopheles stephensi* was greatly affected by the exposure of smoke from *Acalypha indica* and *Acalypha indica*. (Abirami *et al*., 2010). In the present study the smoke emerged from the *C.occidentalis* considerably affected the adult mosquito survival, pronounced high mortality and also treated individual laid minimum number of eggs. The result of this study indicate that *C.occidentalis* leaf extracts enhance the larvicidal and pupicidal activity, the leaves and pods enhances in the smoke repellency test it may be an effective alternative to conventional synthetic insecticides for the control of *A. stephensi.*

We made an attempt to evaluate the *C.occidentalis* for their mosquito larvicidal activity, smoke repellent activity and the results of these experiments have opened the possibility of further investigations on their efficiency, in view of the utilization of their higher biomass. However, the mechanism of action of the flavonoid compounds from *C.occidentalis* is yet unclear. It has therefore necessitated the studies on isolation, purification and mechanism of action of individual compounds existing in the *C.occidentalis* which are in progress.

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